2506-Pos Board B492
Adriamycin-Induced Mitochondrial Toxicity in Rat Heart is Exacerbate by Angiotensin
Eylem Taşkın, Nurcan DURSUN, Elvan Kunduz, Kalender Özdoğan, Mükerrem Betül, Yerer Aycan.
Adriamycin (ADR) increases the production of reactive oxygen species, which diminishes mitochondrial function. Angiotensin-II stimulates mitochondrial-ROS generation. The aim of the study was to examine whether angiotensin converting enzyme (ACE) or renin inhibitors (captopril and/or aliskiren) protect against ADR-induced mitochondrial function impairment.
Rats were divided into five groups (n=14 each). The control group was treated with saline. ADR was administered to the four other groups every 2 days (4 mg/kg i.p). One of these was co-administered captopril (10 mg/kg/daily) and the other was co-treated with aliskiren (50 mg/kg/daily), while another was co-treated with both captopril and aliskiren (captopril and aliskiren were gavage administration daily for 8 days). Left ventricular function, ECG variables and blood pressure were assessed at the end of treatment period. The hearts were homogenized and biochemical measurements were made in mitochondria, cytosol and plasma. Mitochondria membrane potential (MMP), ATP levels were determined.
ADR decreased in the left ventricular developed pressure (LVDP), the maximal rate of rise of pressure (+dP/dt), and increased in the left ventricular end-diastolic pressure (LVEDP). ADR increased ST interval and decreased mean blood pressure. ADR increased oxidative stress in mitochondrial, cytosolic and plasma. ADR decreased MMP and ATP level in myocyte mitochondria. ADR co-administration with renin and ACE inhibitors improved the dissipation of MMP. The decreased in ATP level was restored by treatment with inhibitors of ACE and renin. By maintaining normal levels of mitochondrial MMP and ATP, captopril and aliskiren treatment prevented the pathologic changes in ECG, blood pressure and left ventricular function.
We concluded that inhibitors of angiotensin II are effective against ADR cardiotoxicity via the restoration of MMP and ATP production and prevention of mitochondrial damage in vivo.

Ion Motive ATPases

2507-Pos Board B493
Structural and Physiological Factors Controlling the Ion Specificity of ATP Synthase Membrane Rotors
José D. Faraldo-Gómez, Alexander Krah, Vanessa Leone, Florian Rössler.
ATP synthases are large multi-subunit complexes that utilize the energy stored in transmembrane electrochemical gradients of H(+) or Na(+) for the synthesis of ATP, through a rotary mechanism. A membrane-embedded sub-complex, known as the rotor ring, is the key structural component that transforms the free-energy gained from downhill ion transport into mechanical rotation. The rotor ring thus confers the ATP synthase with its ion specificity, influencing the degree to which cell-growth will be viable in a given physiological context. Here, we review our understanding of the principles that control the ion specificity of ATPase/synthase membrane rotors, based upon extensive theoretical work and biochemical and structural data for a range of representative organisms. In particular, we discuss how a conserved E/D side chain provides the ion-binding sites of all rotors across the F, V and A-ATPase/synthase subfamilies with a strong, intrinsic selectivity for protons. This default specificity, however, is somehow drastically enhanced or reduced to allow for actual H(+) or Na(+) coupling under physiological conditions. We show that such strong modulation is provided by the spectrum of non-conserved amino-acids that decorate the ion-binding sites. While hydrophobic side chains contribute to enhance the H(+) selectivity of the rotors to the extreme levels required in many cases, polar side-chains and structurally-bound water molecules have the opposite effect, and can make a binding site essentially non-specific - which facilitates Na(+)-coupling. Altogether, this analysis illustrates a process of adaptation in the chemical structure of an indispensable enzyme, so as to meet the requirements of a given physiological environment.

2508-Pos Board B494
Interactions Between the γ Subunit and the C-Terminal Domain of the ϵ Subunit Mediate ϵ Subunit Inhibition of F1-ATPase
Natalie C. Negrey, Robert K. Nakamoto.
The ϵ subunit of the F_1 sector of the *Escherichia coli* ATP synthase is part of the rotor complex (γ-ϵ-c_{10}). ϵ subunit plays important roles in energy coupling between H^+ transport and ATP hydrolysis/synthesis but also imparts an inhibitory activity on the catalytic mechanism. We characterize the structure/function effects of two γ subunit Cys substitutions that affect ϵ inhibition of ATPase activity. γT106C by itself has little affect, but labeling of the cysteine with methanethiosulfonate spin label (MTSL) blocks the ability of exogenously added ϵ subunit to inhibit the enzyme. In contrast, MTSL labeling of the ϵ-replete F_1 remains inhibited. We hypothesize that a bulky hydrophobic adduct of γT106 blocks proper interactions with the ϵ subunit. The inhibition of ATPase activity by ϵ is abrogated in the γE224C mutant. However, unlike the wild-type enzyme, which has significantly higher activation energy, E_A, for steady state ATPase activity, γE224C mutant F_1 has the same E_A as the ϵ-replete WT enzyme. The results indicate that efficient energy coupling of the γ subunit mutant enzyme is retained. Crystal structure of the E. coli γ-ϵ dimer (Rogers, A. J. W. and Wilce, M. C. J., *Nat. Struct. Biol.* 7, 1051-1054, 2000) suggests that replacement of γThr106 and γGlu224 may disrupt interactions with the C-terminal helix-turn-helix domain of the ϵ subunit. Our data suggest that the ϵ subunit carboxyl terminal domain is responsible for inhibition of catalytic activity also but also plays a role in mediating proper interactions between the γ and *c* subunits and efficient coupling.

2509-Pos Board B495
Unravelling the Symmetry Mismatch of the Two Coupled Rotary Motors of a Single F_OF_1-ATP Synthase by Three Color Fret
Michael Börsch.
F_oF_1-ATP synthase is the enzyme that provides the 'chemical energy currency' adenosine triphosphate, ATP, for living cells. The enzyme consists of the membrane-embedded F_o part and the F_1 part with three catalytic nucleotide binding sites. The formation of ATP is accomplished by a stepwise internal rotation of subunits within both parts. During ATP synthesis, proton translocation through the F_o part drives a 10-step rotary motion of the c_{10} ring with respect to the non-rotating subunits *a* and b_2 [1]. This rotation is transmitted to the γ and ϵ subunits of the F_1 part which perform three 120° steps per full rotation [2]. To localize transient elastic energy storage within the enzyme due to the symmetry mismatch, we monitor subunit rotation by a single-molecule fluorescence resonance energy transfer (FRET) approach using three fluorophores specifically attached to the enzyme. Cy5 is attached to one *c* subunit and EGFP to the *a* subunit of the F_o part [3]. The F_1 part has a fluorophore bound at the ϵ subunit. To reduce photophysical artifacts due to spectral fluctuations of the fluorophores, a duty cycle-optimized alternating three laser scheme (DCO-ALEX) has been developed. Simultaneous observation of the stepsizes for both motors in a single enzyme revealed elastic deformations in the rotor parts of F_o and F_1 during catalysis.
[1] M. G. Düser, N. Zarrabi, D. J. Cipriano, S. Ernst, G. D.Glick, S.D. Dunn, M. Börsch (2009) EMBO Journal 28, 2689-2696.
[2] B. Zimmermann, M. Diez, N. Zarrabi, P. Gräber, M. Börsch (2005) EMBO Journal 24, 2053-2063.
[3] M. G. Düser, Y. Bi, N. Zarrabi, S.D. Dunn, M. Börsch (2008) J. Biol. Chem. 283, 33602-33610.

2510-Pos Board B496
Revisiting Boyer's Hydronium Hypothesis: On the Mechanism of Proton Binding to ATP Synthase Membrane Rotors
Vanessa Leone, Alexander Krah, José D. Faraldo-Gómez.
A recently determined atomic structure of an H^+-transporting ATP synthase membrane rotor has revived the long-standing question of whether protons may be bound to these structures in the form of a hydronium ion. Using both classical and quantum-mechanical simulations, we show that this notion is implausible. *Ab initio* molecular dynamics simulations of the binding site demonstrate that the putative H_3O^+ deprotonates within femtoseconds. The bound proton is thus transferred irreversibly to the carboxylate side chain found in the ion-binding sites of all ATP synthase rotors. This result is consistent with classical simulations of the rotor in a phospholipid membrane, on the 100-nanosecond time-scale. These simulations show that the hydrogen-bond network seen in the crystal structure is incompatible with a bound hydronium. The observed coordination geometry is shown to correspond instead to a protonated carboxylate and a bound water molecule. In conclusion, this study underscores the notion that binding and transient storage of protons in the membrane rotors of ATPases/synthases occur through a common chemical mechanism, namely carboxylate protonation.

2511-Pos Board B497
Side-Chain Carboxylate Needed for Nonstoichiometric Proton Leak through Na/K-ATPase Pumps
Natascia Vedovato, David C. Gadsby.
Cyclic, stoichiometric exchange of 3 Na ions for 2 K ions per ATP hydrolyzed generates outward Na/K-ATPase pump current. With no external Na (Nao) and K (Ko), a nonstoichiometric inwardly-rectifying current flows through Na/K-pumps, proposed carried by protons because it is increased when pH is lowered. Stoichiometric and nonstoichiometric currents are both abolished by the Na/K-pump inhibitor ouabain. In *Xenopus* pumps with ouabain-resistance mutation C113Y, nonstoichiometric current appeared even at pH 7.6 in the absence of Nao and Ko, was augmented ~4 fold at pH 6, was abolished by Ko, and was diminished by high Nao. We suggested that alternate-side exposure of